UPLC and ESI-MS analysis of metabolites of *Rauvolfia tetraphylla* L. and their spatial localization using desorption electrospray ionization (DESI) mass spectrometric imaging

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Identification of indole alkaloids using exact mass and ESI MS/MS analysis

ESI MS analysis of the different parts (root, stem, leaf and fruits) of *R. tetraphylla* showed the presence of prominent metabolite signatures in the range of *m/z* 150-1000 **(Table1).** Among the different tissues, roots contained relatively large number of indole alkaloids (Fig S2). Structural characterization of these indole alkaloids were done by both mass fragmentation analysis and accurate mass measurement **(Fig S2)** (Smith et al., 2005). A list of *m/z* values of the parent and fragment ions and their chemical formulae are given in **Table 1.**

Based on the masses obtained, we present below the salient results pertaining to exact masses and to the fragmentation ions.

Strictosidine ($C_{27}H_{34}N_2O_9$: exact mass *m/z* 531.2337), a key metabolic precursor in the synthesis of indole alkaloids was identified by ESI MS analysis in root and leaf. The identity was also confirmed by the characteristic fragmentions: at *m/z*514 due to loss of H_2O, *m/z* 499 due to loss of CH_3OH, *m/z* 351 is due loss of glucose, *m/z* 222, 223 and *m/z* 144 is due to breakage of C ring. These fragmentations corresponded with the published literatures and metabolite databases (Yamamoto et al., 2016) **(Fig S2a).**Here we present briefly the major alkaloids represented under three broad categories, namely, reserpine type, ajmalcine type and serpentine type of alkaloids.

Reserpine type of alkaloids

Yohimbine ($C_{21}H_{26}N_2O_3$), *m/z* 355.2 and exact mass of *m/z* 355.2007, has a pentacyclic ring system with substitution at C16 and C17. The MS/MS spectrum showed five characteristic fragment ions at *m/z* 337, 323, 224, 212 and 144. The product ions at *m/z* 323 and 337 were produced due to the loss of CH_3OH and H_2O in terpene moiety, whereas the product ions at *m/z* 224, 212 and 144 produced due to cleavage of C ring **(Fig S2b)**. Correspondingly, the ion at *m/z* 609.2 and exact mass *m/z* 609.2802 was identified as reserpine ($C_{33}H_{41}O_9N_2$). ESI MS fragmentation pattern of m/z 609.2 showed the characteristic fragment ions at *m/z* 397.2 due to loss of trimethoxybenzoic acid moiety and *m/z* 195.0 due to cleavage of ester bond between trimethoxybenzoic acid from the rest of moiety. Further, fragment ion at *m/z* 397.2 fragmented to m/z 365.2 due to loss of methanol, and *m/z* 236.01 due to loss of methanol followed by cleavage of C-ring. Fragment ion at *m/z* 448.2 was also produced due to cleavage of C ring from the precursor ion **(Fig S2c)**. The ion at *m/z* 144 showed the presence of indole moiety fragmentation, this is one of the characteristic fragment confirming the molecule is the indole group of compounds (Bindu et al., 2014; Kumar et al., 2016a).

Similarly, few other reserpine like molecules *m/z* 341.2, 371.2, 565.2, 579.2 were identified as yohimbinic acid, 18-hydroxyepialloyohimbine, raunescine, deserpidine

respectively based on the exact mas and their mass fragmentation **(Fig S2d-i).** Yohimbinic acid (*m/z* 341.1862; $C_{20}H_{25}N_2O_3$: **Fig S2d)** mass fragmented to *m/z* 323 due to the loss of H_2O in terpene moiety, whereas the product ions at *m/z* 196, 212 and 144 produced due to cleavage of C ring **(Fig S2b).** 18-hydroxyepialloyohimbine (*m/z*371.1961; $C_{21}H_{26}N_2O_4$: **Fig S2e)** fragmented to *m/z* 353 and 339, were due to the loss of CH_3OH and H_2O in terpene moiety, whereas the product ions at *m/z* 240, 228 and 144 produced due to cleavage of C ring **(Fig S2c).** Deserpidine (*m/z* 579.2698; $C_{32}H_{38}N_2O_8$: **Fig S2 f-g**) and raunescine (*m/z* 565.2542: $C_{31}H_{36}N_2O_8$: **Fig S2h-i**) and showed the characteristic mass fragmentations similar to reserpine, *m/z* 321.2 and *m/z* 367.2 due to loss of trimethoxybenzoic acid moiety and *m/z* 195.0 due to cleavage of ester bond between trimethoxybenzoic acid from the rest of moiety. Fragment ion at *m/z* 448.2 was also produced due to cleavage of C ring from the precursor ion, respectively **(Fig S2 d-i).** Further, fragment ion of deserpidine at *m/z* 397.2 fragmented to *m/z* 365.2 due to loss of methanol, and *m/z* 236.01 due to loss of methanol followed by cleavage of C-ring **(Fig S2d-i)** (Kumar et al., 2016a; Kumar et al., 2016b; Pandey et al., 2016).

Ajmalcine type of alkaloids

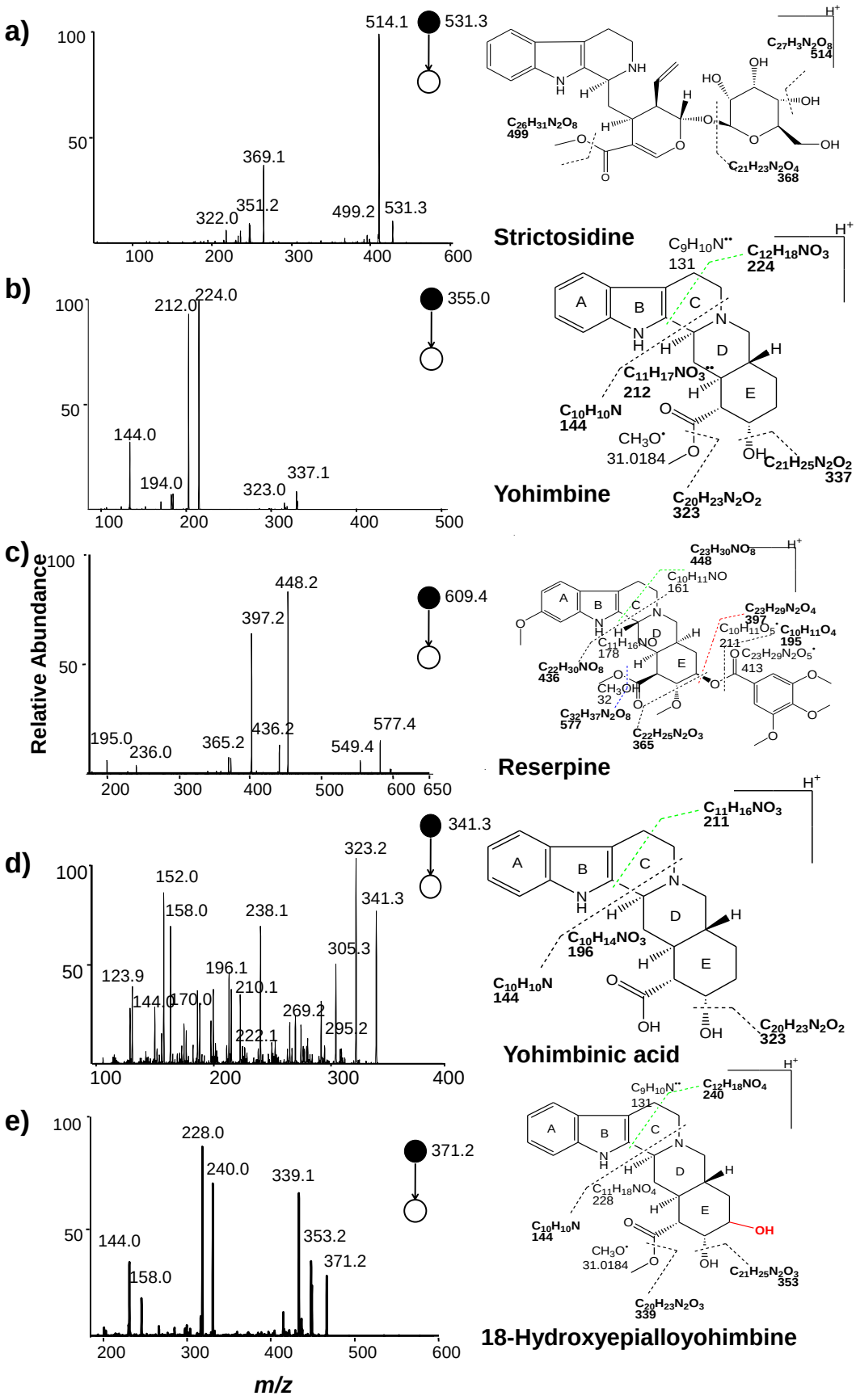
The ion at *m/z* 353.2 was identified as ajmalcine (*m/z* 353.1851; $C_{21}H_{25}N_2O_3$: **Fig S2j).** The product ions at *m/z* 321 was produced due to the loss of CH_3OH in terpene moiety, where as the product ions at *m/z* 210, 222 and 144 were produced due to cleavage of C ring **(Fig S2j-k).** Similarly, *m/z* 413.2 identified as reserpiline (*m/z* 413.2068; $C_{23}H_{29}N_2O_5$: **Fig S2k**. The mass fragmentation of *m/z* 413.2 showed five characteristic fragment ions at *m/z* 337, 224, 204 and 144. The product ions at *m/z* 339 produced due to the loss of CH_3OH in terpene moiety, whereas the product ions at *m/z* 224, 204 and 144 produced due to cleavage of C ring **(Fig S2i-k)** (Bindu et al., 2014; Kumar et al., 2015; Kumar et al., 2016b; Kumar et al., 2016c; Pandey et al., 2016; Sagi et al., 2016).

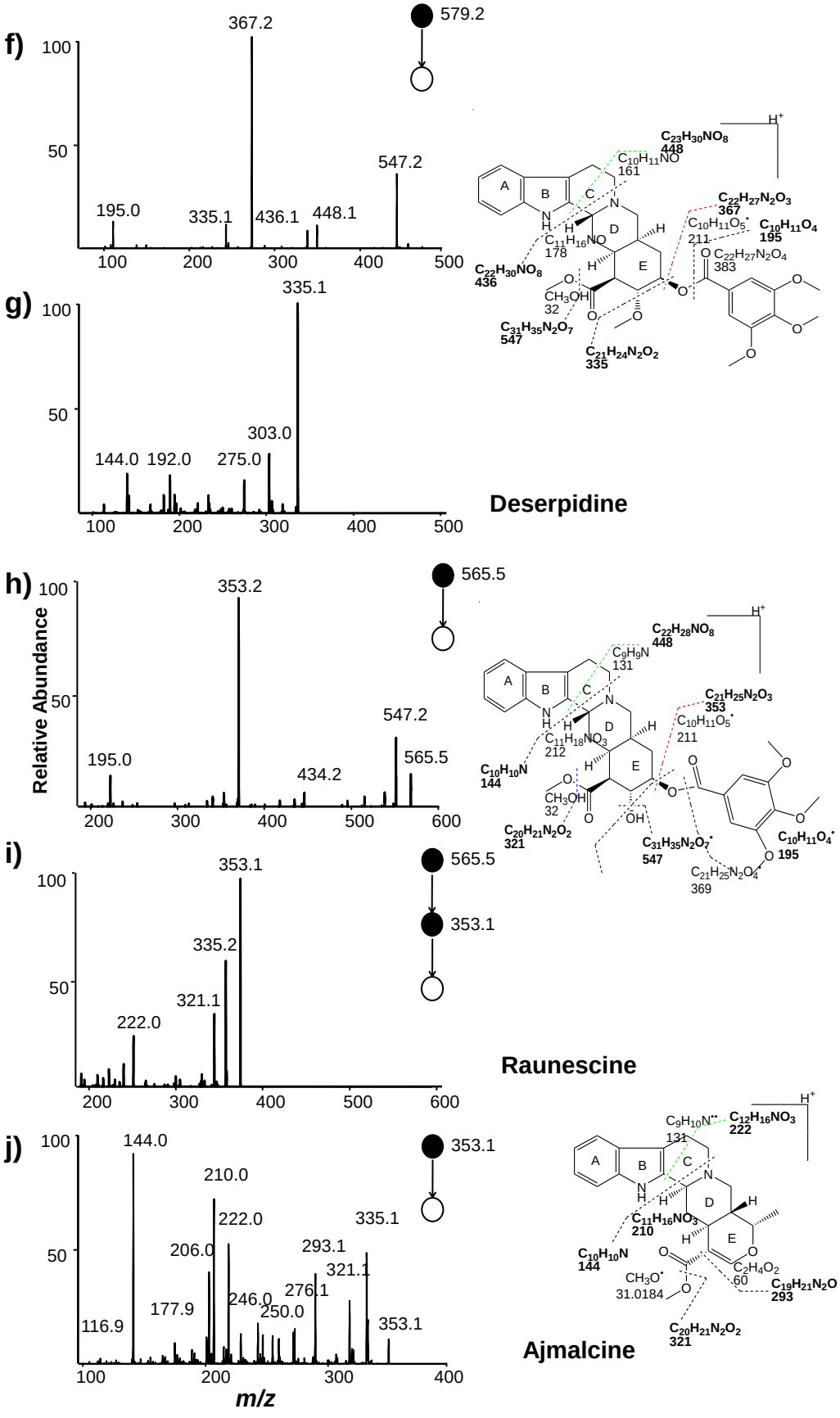
Ajmaline type of alkaloids

The ion *m/z* 327.2 at ajmaline **(***m/z* 327.2063; $C_{20}H_{27}N_2O_2$: **Fig S2l**) showed the fragment ions at *m/z* 309, 238, 210, 194,182, 158, 144**.** The product ions at *m/z* 309 and *m/z* 291 were produced due to the successive losses of H_2Oin terpene moiety, whereas the product ions at *m/z* 238, 212, 194 and 144 were produced due to cleavage of C ring. Methyl indole derivative (*m/z* 158) is formed by ring cleavage at C-ring and further loss of methyl group from nitrogen atom yields the base peak at *m/z* 144 $[C_{10}H_{10}N]^+$. Loss of C_8H_7NO from protonated ion gives *m/z* 194 $[C_{12}H_{21}NO]^+$**(Fig S2l-q).** Similarly, ions at *m/z* 309.2, 311.1, 313.2, 323.2, 325.2 identified as sarpagine (*m/z* 311.175; $C_{19}H_{23}N_2O_2$: **Fig S2m**), 10-O-methylsarpagine (*m/z* 325.1907; $C_{20}H_{25}N_2O_2$: **Fig S2n-o**, tetraphyllicine (*m/z* 309.1957: $C_{20}H_{25}N_2O$: **Fig S2p**), mitoridine (*m/z* 323.1749; $C_{20}H_{23}N_2O_2$: **Fig S2q)** respectively. Identity of these

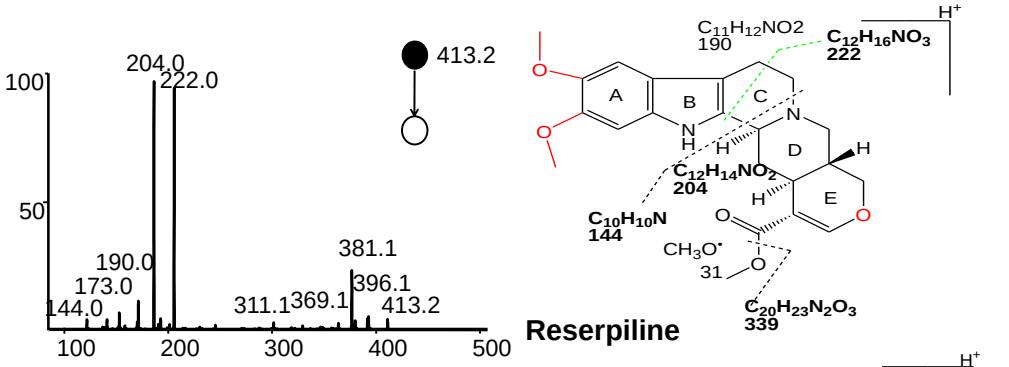
molecules was assigned based on the fragmentation patterns at C-ring cleavage **(Fig S2m-q)** (Bindu et al., 2014; Kumar et al., 2015; Kumar et al., 2016b; Kumar et al., 2016c; Pandey et al., 2016).

The ions at *m*/*z* 349.1 were identified as serpentine (*m*/*z* 349.1537; $C_{21}H_{20}N_2O_3$). Serpentine showed the characteristic fragment ions at *m*/*z* 317, 289, 277 and 263. The product ions at *m*/*z* 317, 289 produced were due to the loss of CH_3OH and CO respectively, in terpene moiety, whereas the product ions at *m*/*z* 277 and 263 produced were due to cleavage of E ring. One of the derivative of serpentine, demethyl serpentine was identified based on similar fragmentations **(Fig S2s-u)** (Kumar et al., 2016b).


a)
514.1
531.3
369.1
322.0
351.2
499.2
531.3
Strictosidine
b)
212.0
224.0
355.0
144.0
194.0
323.0
337.1
Yohimbine
c)
Relative Abundance
448.2
397.2
609.4
195.0
236.0
365.2
436.2
549.4
577.4
Reserpine
d)
341.3
323.2
152.0
158.0
238.1
305.3
123.9
144.0
170.0
196.1
210.1
269.2
222.1
295.2
Yohimbinic acid
e)
371.2
228.0
240.0
339.1
144.0
158.0
353.2
371.2
18-Hydroxyepialloyohimbine
m/z

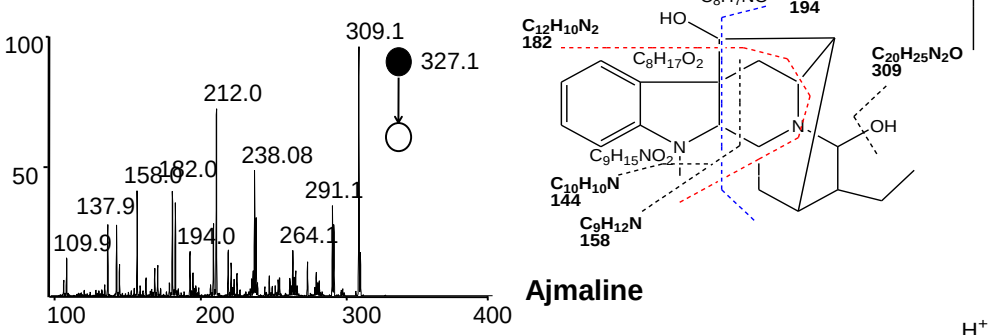

f)
579.2
367.2
547.2
195.0
335.1
436.1
448.1
g)
335.1
303.0
144.0
192.0
275.0
Deserpidine
h)
565.5
353.2
547.2
195.0
434.2
565.5
Relative Abundance
i)
565.5
353.1
353.1
335.2
321.1
222.0
Raunescine
j)
353.1
144.0
210.0
222.0
335.1
206.0
293.1
321.1
276.1
246.0
177.9
250.0
116.9
353.1
m/z
Ajmalcine

k)



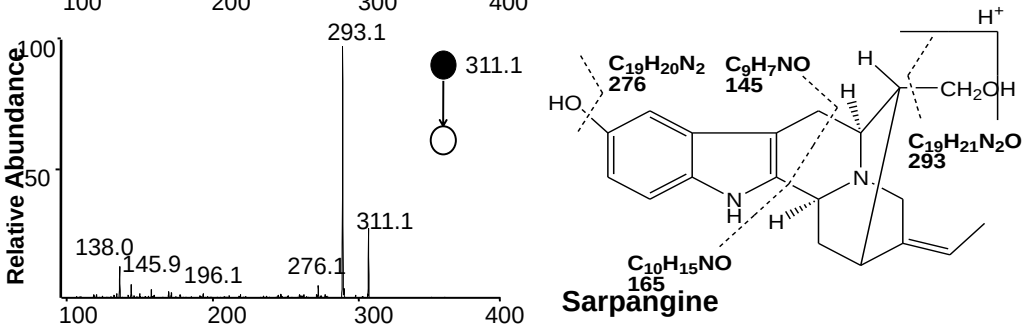
Reserpiline

l)



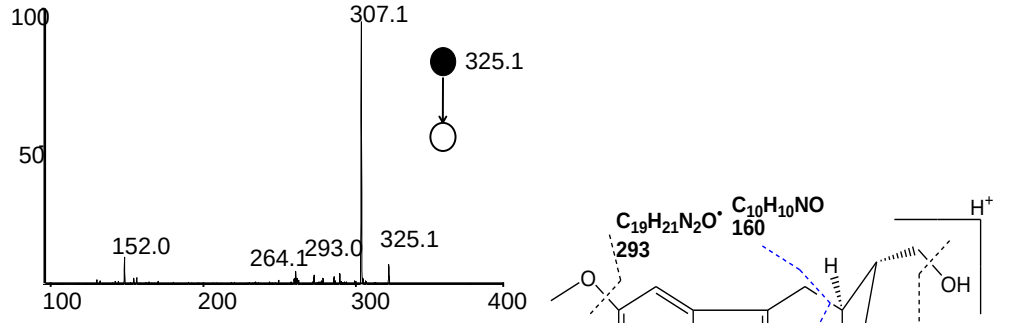
Ajmaline

m)

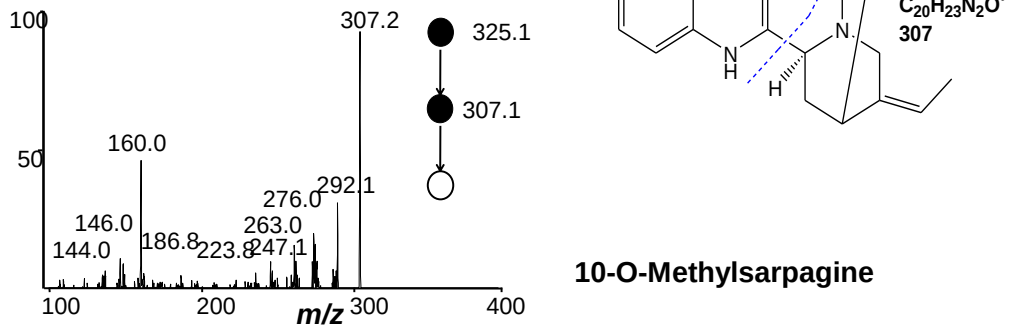


Sarpangine

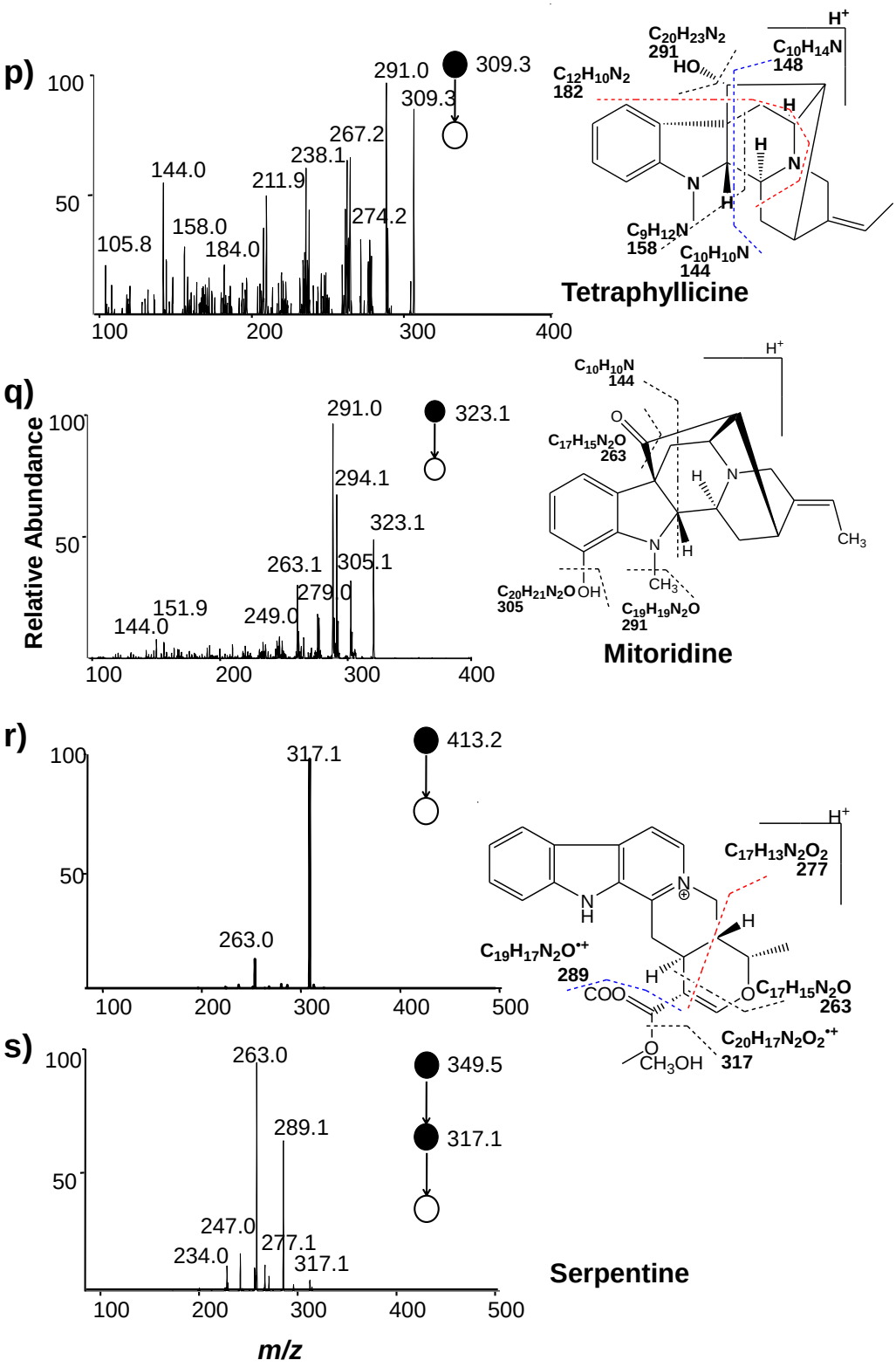
n)



o)



10-O-Methylsarpagine


p)
100
50
105.8
144.0
158.0
184.0
211.9
238.1
267.2
274.2
291.0
309.3
309.3
100
200
300
400
H+
C20H23N2
291
HO
C10H14N
148
C12H10N2
182
H
H
N
N
H
C9H12N
158
C10H10N
144
Tetraphyllicine
q)
Relative Abundance
100
50
144.0
151.9
249.0
263.1
279.0
291.0
294.1
305.1
323.1
323.1
100
200
300
400
H+
C10H10N
144
O
C17H15N2O
263
H
N
N
H
CH3
C20H21N2O OH
305
CH3
C19H19N2O
291
Mitoridine
r)
100
50
263.0
317.1
413.2
100
200
300
400
500
H+
C17H13N2O2
277
N
H
N⊕
H
C19H17N2O•+
289
H
COO
O
C17H15N2O
263
O
CH3OH
C20H17N2O2•+
317
s)
100
50
234.0
247.0
263.0
277.1
289.1
317.1
349.5
317.1
100
200
300
400
500
m/z
Serpentine

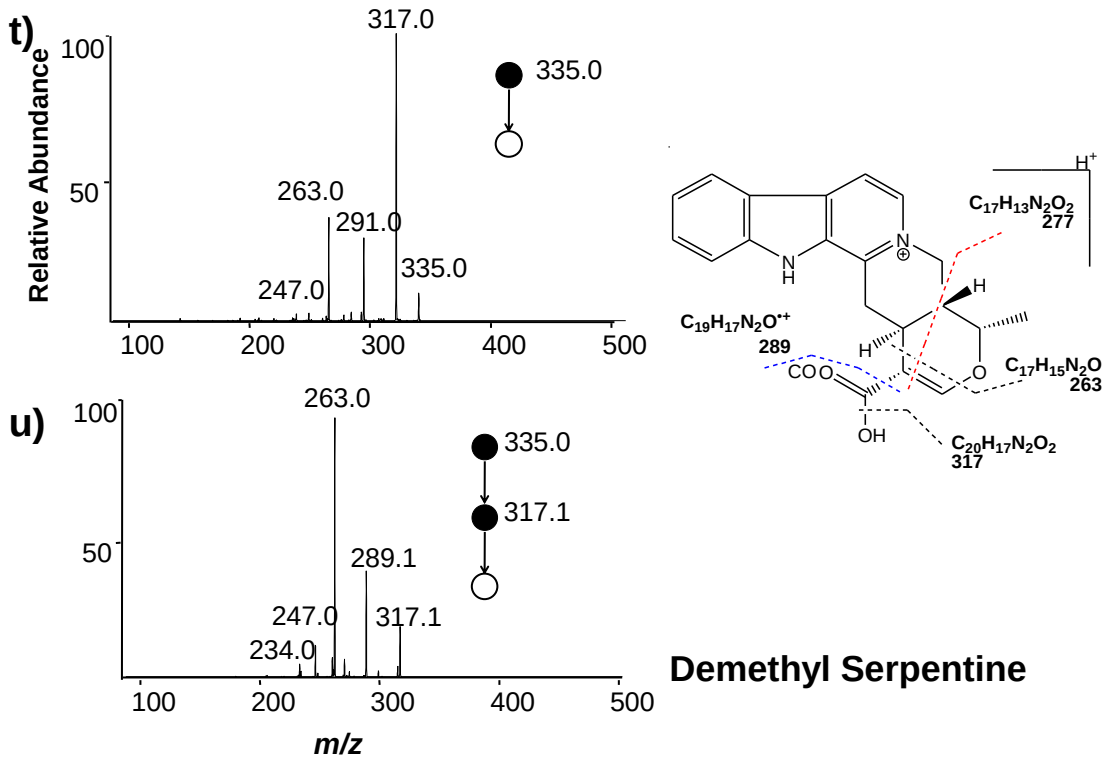


Fig S2. ESI MS/MS analysis of monoterpenoid indole alkaloids and their respective chemical structure and mass fragmentation patterns.

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